CLAIMS

What is claimed is:

1. A polynucleotide comprising a nucleotide sequence that encodes a chimeric polypeptide, said polynucleotide comprising:

a nucleotide sequence encoding at least one SCHAG amino acid sequence fused in frame with a nucleotide sequence encoding at least one polypeptide of interest other than a marker protein, a glutathione S-transferase (GST) protein, or a Staphylococcal nuclear protein.

2. A polynucleotide according to claim 1 wherein the at least one SCHAG amino acid sequence comprises at least one prion-aggregation domain of a prion protein.

3. A polynucleotide according to claim 2, further comprising a nucleotide sequence encoding a translation initiation codon and a secretory signal peptide fused in frame and upstream of the encoding sequences.

4. A polynucleotide according to claim 2, further comprising a translation initiation codon fused in frame and upstream (5') of the encoding sequences, and a translation stop codon fused in frame and downstream (3') of the encoding sequences.

5. A polynucleotide according to claim 4 wherein said polynucleotide further includes a sequence encoding an endopeptidase or chemical recognition sequence fused in frame between the sequence encoding the at least one prion-aggregation domain and the sequence encoding the polypeptide of interest.

6. A polynucleotide according to claim 4 wherein the nucleotide sequence encoding the at least one prion-aggregation domain is fused upstream (5') of the sequence encoding the at least one polypeptide of interest.

7. A polynucleotide according to claim 4 further comprising a promoter sequence operatively connected upstream (5') of the encoding sequences.

8. A polynucleotide according to claim 7 further comprising a polyadenylation signal sequence operatively connected downstream (3') of the encoding sequences.

9. A polynucleotide according to claim 4, wherein the polynucleotide further includes a sequence encoding a selectable marker protein.

10. A polynucleotide according to claim 4, wherein the at least one prion-aggregation domain comprises the prion aggregation domain of a protein selected from the group consisting of: mammalian prion proteins (PrP) and Ht proteins; Sup35 proteins; Ure2 proteins; and Rnq1 proteins.

11. A polynucleotide according to claim 4 wherein the at least one prion-aggregation domain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 17, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 46, 47, and 50 and prion aggregation domain fragments thereof.

12. A polynucleotide according to claim 4, wherein the at least one prion-aggregation domain comprises the amino acid sequence of positions 2-113 of SEQ ID NO: 2.

13. A polynucleotide according to claim 4, wherein the at least one prion-aggregation domain comprises the amino acid sequence of positions 2-65 of SEQ ID NO: 4.

14. A polynucleotide according to claim 4 wherein the at least one polypeptide of interest is an enzyme.

15. A polynucleotide according to claim 4 wherein the at least one polypeptide of interest is a polypeptide capable of binding a composition of interest.

16. A polynucleotide according to claim 4 wherein the at least one polypeptide of interest comprises at least one antigen binding domain of an antibody.

17. A polynucleotide according to claim 4 wherein the at least one polypeptide of interest comprises at least one ligand binding domain of a ligand binding protein.

18. A polynucleotide according to claim 4, wherein the at least one polypeptide of interest comprises a ligand of a cell surface receptor.

19. A host cell transformed or transfected with a polynucleotide according to claim 4.

20. A vector comprising a polynucleotide according to claim 4.

21. A host cell transformed or transfected with a vector according to claim 20.

22. A polynucleotide comprising a nucleotide sequence that encodes a chimeric polypeptide, said chimeric polypeptide comprising an amyloidogenic domain that causes the polypeptide to aggregate with identical polypeptides into fibrils, fused to a domain comprising a polypeptide of interest;

wherein the amyloidogenic domain comprises an amyloidogenic amino acid sequence of a naturally occurring protein and further includes a duplication of at least a portion of said naturally occurring amyloidogenic amino acid sequence, said duplication increasing the amyloidogenic affinity of said chimeric polypeptide relative to an identical chimeric polypeptide lacking said duplication.

23. A polynucleotide according to claim 22 wherein the naturally occurring protein comprises a Sup35 protein of *Saccharomyces cereviciae* characterized by the partial amino acid sequence PQGGYQQYN, and wherein said duplication includes the amino acid sequence PQGGYQQYN.

24. A polynucleotide comprising a nucleotide sequence that encodes a chimeric polypeptide, said chimeric polypeptide comprising an amyloidogenic domain that causes the polypeptide to aggregate with identical polypeptides into fibrils, fused to a domain comprising a polypeptide of interest; wherein the amyloidogenic domain comprises amyloidogenic amino acid sequences of at least two naturally occurring amyloidogenic proteins.

25. A polynucleotide encoding a chimeric polypeptide, said polypeptide comprising at least two prion-aggregation domains fused in frame with at least one polypeptide of interest.

26. A chimeric polypeptide encoded by a polynucleotide of claim 1.

27. A composition comprising a purified polypeptide according to claim 26.

28. A chimeric polypeptide encoded by a polynucleotide of claim 22.

29. A chimeric polypeptide encoded by a polynucleotide of claim 24.

30. A chimeric polypeptide encoded by a polynucleotide of claim 25.

31. A fibril comprising an ordered aggregate of chimeric polypeptides according to claim 26.

32. A composition comprising at least one polypeptide aggregate, said polypeptide aggregate comprising a plurality of chimeric polypeptides according to claim 26.

33. A composition according to claim 32 wherein said polypeptide aggregate is insoluble in water.

34. A method comprising the steps of:

transforming or transfecting a cell with a polynucleotide according to claim 1; and

growing the cell under conditions which result in expression of said chimeric polypeptide in said cell.

35. A method according to claim 34, further comprising the step of isolating the chimeric polypeptide from the cell or from growth medium of the cell.

36. A method according to claim 35, further comprising the step of proteolyically detaching the SCHAG amino acid sequence of the protein from the polypeptide of interest.

37. A method according to claim 36, further comprising the step of isolating the protein of interest from the SCHAG amino acid sequence.

38. A method of making a protein of interest, comprising the steps of:

transforming or transfecting a cell with a polynucleotide, said polynucleotide comprising a nucleotide sequence that encodes a chimeric polypeptide, said chimeric polypeptide comprising an amyloidogenic domain that causes the polypeptide to aggregate with identical polypeptides into fibrils, fused to domain comprising a polypeptide of interest;

growing the cell under conditions which result in expression of said chimeric polypeptide in said cell and aggregation of said chimeric polypeptide into fibrils; and

isolating the chimeric polypeptide from the cell or from growth medium of the cell.

39. A method according to claim 38 wherein said isolating step comprises the step of separating the fibrils from soluble proteins of said cell.

40. A method according to claim 39, further comprising the steps of proteolytically detaching the amyloidogenic domain of the chimeric protein from the polypeptide of interest; and isolating the polypeptide of interest.

41. A method of modifying a living cell to create an inducible and stable phenotypic alteration in the cell, comprising the step of transforming or transfecting a living cell with a polynucleotide according to claim 7, wherein the promoter sequence of said polynucleotide promotes expression of the chimeric polypeptide in the cell and is inducible to promote increased expression of the chimeric polypeptide to a level that induces aggregation of the chimeric polypeptide into fibrils.

42. A method according to claim 41, further comprising the step of growing the cell under conditions which induce the promoter, thereby causing increased expression of the polypeptide and inducing aggregation of the chimeric polypeptide into fibrils in the cell.

43. A method according to claim 42 wherein the SCHAG amino acid sequence comprises an amino terminal domain of a Sup35 protein.

44. A method according to claim 43 wherein the host cell is a yeast cell that comprises a mutant Sup35 gene that expresses a Sup35 protein lacking an amino terminal domain capable of prion aggregation.

45. A method for reverting the phenotype obtained according to the method of claim 42, comprising the step of overexpressing a chaperone protein in the cell to convert the polypeptide from a fibril-forming conformation into a soluble conformation.

46. A polynucleotide useful for performing homologous recombination in a living cell to convert a protein-encoding gene of the cell to a prion gene of the cell, said polynucleotide comprising a nucleotide sequence of the formula FPBT or FBPT, wherein:

B comprises a nucleotide sequence encoding a polypeptide that is encoded by a portion of the genome of the cell;

F and T comprise, respectively, 5' and 3' flanking sequences adjacent to the sequence encoding B in the genome of the cell; and

P comprises a nucleotide sequence encoding a prion-aggregation amino acid sequence, wherein P is fused in frame to B.

47. A method of modifying a living cell to create an inducible and stable phenotypic alteration in the cell, comprising the steps of:

transforming a living cell with a polynucleotide according to claim 46;

culturing the cell under conditions that permit homologous recombination between said polynucleotide and the genome of the cell; and

selecting a cell in which said polynucleotide has homologously recombined with the genome to create a genomic sequence comprising the formula PB or BP.

48. A method of modifying a living cell to create an inducible and stable phenotypic alteration in the cell, comprising steps of:

identifying a target polynucleotide sequence in the genome of the cell that encodes a polypeptide of interest; and

transforming the cell to substitute for or modify the target sequence, wherein the substitution or modification produces a cell comprising a polynucleotide that encodes a chimeric polypeptide, wherein the chimeric polypeptide comprises a SCHAG amino acid sequence fused in frame with the polypeptide of interest.

49. A composition comprising an ordered aggregate of at least two chimeric polypeptides according to claim 1, said at least two chimeric polypeptides having compatible SCHAG amino acid sequences and distinct polypeptides of interest.

50. A composition according to claim 49 wherein the at least two chimeric polypeptides comprise identical SCHAG amino acid sequences.

51. A composition according to claim 49 wherein the ordered aggregate comprises a fiber and wherein the polypeptides of interest retain native biological activity.

52. A host cell transformed or transfected with at least two polynucleotides according to claim 1, wherein said two polynucleotides comprise compatible SCHAG amino acid sequences and distinct polypeptides of interest.

53. A cell culture comprising cells transformed or transfected with a polynucleotide according to claim 1, wherein the cells express the chimeric polypeptide encoded by the polynucleotide, and wherein the cell culture includes cells wherein said chimeric polypeptide is present in an aggregated state and cells free of aggregated chimeric polypeptide.

54. A cell culture according to claim 53, wherein at least some cells convert between a phenotype characterized by aggregated chimeric polypeptide and a phenotype characterized by both the presence of unaggregated chimeric polypeptide and the absence of aggregated chimeric polypeptide.

55. A method of making a reactable SCHAG amino acid sequence, comprising the steps of:

(a) identifying a SCHAG amino acid sequence, wherein polypeptides comprising the SCHAG amino acid sequence are capable of forming ordered aggregates;

(b) analyzing the SCHAG amino acid sequence to identify at least one amino acid residue in the sequence having an amino acid side chain that is exposed to the environment in an ordered aggregate of polypeptides that comprise the SCHAG amino acid sequence; and

(c) modifying the SCHAG amino acid sequence by substituting an amino acid containing a reactive side chain for the at least one amino acid identified according to step (b), thereby making a reactable SCHAG amino acid sequence.

56. A method according to claim 55, further comprising a step (d) of making a polypeptide comprising the reactable SCHAG amino acid sequence.

57. A method according to claim 56, further comprising a step (e) of making a polymer comprising an ordered aggregate of polypeptide monomers, where at least one of the polypeptide monomers comprises the reactable SCHAG amino acid sequence, and wherein the reactive side chains of the monomers that comprise the reactable SCHAG amino acid sequence are exposed to the environment in the polymer.

58. A method according to claim 57, further comprising a step (f) of contacting the reactive side chains with a chemical agent to attach a substituent to the reactive side chains.

59. A method according to claim 58, wherein the substituent comprises a member selected from the group consisting of: an enzyme; a metal atom; an affinity binding molecule having a specific affinity binding partner; a carbohydrate; a fluorescent dye; a chromatic dye, an antibody; a growth factor; a hormone; a cell adhesion molecule; a toxin; a detoxicant; and a catalyst.

60. A method according to claim 58, wherein the substituent comprises a metal atom.

61. A method according to claim 58 wherein the substituent comprises a fluorescent dye.

62. A method according to claim 56, further comprising steps of:

(e) contacting polypeptides comprising the reactive side chains with a chemical agent to attach a substituent to the reactive side chains, thereby providing modified polypeptides; and

(f) making a polymer comprising an ordered aggregate of polypeptide monomers, wherein at least one of the polypeptide monomers comprise the modified polypeptides.

63. A method according to claim 55, further comprising steps of:

(d) analyzing the SCHAG amino acid sequence to identify at least a second amino acid residue in the sequence having an amino acid side chain that is exposed to the environment in an ordered aggregate of polypeptides that comprise the SCHAG amino acid sequence; and

(e) modifying the SCHAG amino acid sequence by substituting an amino acid containing a reactive side chain for at least one amino acid identified according to step (d); wherein the amino acids substituted in steps (c) and (e) differ, thereby making a reactable SCHAG amino acid sequence with at least two selectively reactable sites.

64. A method according to claim 63, further comprising a step (f) of making a polypeptide comprising the reactable SCHAG amino acid sequence with at least two selectively reactable sites.

65. A polypeptide comprising a reactable SCHAG amino acid sequence made according to the method of claim 56.

66. A polynucleotide comprising a nucleotide sequence that encodes a polypeptide according to claim 65.

67. A polymer comprising polypeptide subunits coalesced into ordered aggregates, wherein at least one of the polypeptide subunits comprises a reactable SCHAG amino acid sequence made according to the method of claim 55.

68. A polymer comprising polypeptide subunits coalesced into ordered aggregates, wherein at least 0.1 % of the polypeptide subunits comprises a reactable SCHAG amino acid sequence according to claim 55.

69. A polymer comprising polypeptide subunits coalesced into ordered aggregates, wherein at least 1 % of the polypeptide subunits comprises a reactable SCHAG amino acid sequence according to claim 55.

70. A polymer comprising polypeptide subunits coalesced into ordered aggregates, wherein at least 10 % of the polypeptide subunits comprises a reactable SCHAG amino acid sequence according to claim 55.

71. A polymer comprising polypeptide subunits coalesced into ordered aggregates, wherein at least 50 % of the polypeptide subunits comprises a reactable SCHAG amino acid sequence according to claim 55.

72. A method according to claim 55, wherein the amino acid containing a reactive side chain according to step (c) is selected from the group consisting of cysteine, lysine, tyrosine, glutamate, aspartate, and arginine .

73. A method according to claim 55, wherein the amino acid containing a reactive side chain according to step (c) is cysteine.

74. A method according to claim 55, wherein the amino acid containing a reactive side chain according to step(c) is lysine.

75. A method of making a fiber with a predetermined quantity of reactive sites for chemically modifying the fiber, comprising the steps of:

(a) providing a first polypeptide comprising a first SCHAG amino acid sequence that is capable of forming ordered aggregates with polypeptides identical to the first polypeptide;

(b) providing a second polypeptide comprising a second SCHAG amino acid sequence that is capable of forming ordered aggregates with polypeptides identical to the first polypeptide or the second polypeptide, wherein the second SCHAG amino acid sequence includes at least one amino acid residue having a reactive amino acid side chain that is exposed to the environment and serves as a reactive site in ordered aggregates of the second polypeptide; and

(c) mixing the first and second polypeptides under conditions favorable to aggregation of the polypeptides into ordered aggregates, wherein the polypeptides are mixed in quantities selected to provide a predetermined quantity of second polypeptide reactive sites.

76. A fiber made by the process of claim 75.

77. A method according to claim 75, further comprising a step (d) of reacting the reactive side chains to attach a substituent to the reactive amino acid side chains of the fiber.

78. A method according to claim 75, wherein the reactive side chains of the fiber are reacted to attach a substituent before step (c).

79. A fiber made by the process of claim 77 or 78.

80. A method according to claim 75, wherein the first SCHAG amino acid sequence includes at least one amino acid residue having a reactive amino acid side chain that is exposed to the environment and serves as a reactive site, and wherein the reactive amino acid side chains of the first and second SCHAG amino acid sequences that are exposed to the environment in ordered aggregates are not identical, thereby permitting selective reaction of the reactive amino acid side chain of the first SCHAG amino acid sequence without reacting the reactive amino acid side chain of the second SCHAG amino acid sequence.

81. A purified polypeptide comprising an amino acid sequence that includes a SCHAG amino acid sequence and at least two amino acid residues having reactive amino acid side chains that are exposed to the environment and serve as reactive sites in ordered aggregates of the polypeptide.

82. A purified polypeptide according to claim 81, wherein the at least two amino acids comprise different, selectively reactable amino acid side chains.

83. A polypeptide comprising a SCHAG amino acid sequence selected from the group consisting of: SEQ ID NOS: 2, 4, and 50, or fragments thereof, with the proviso that at least one amino acid in the SCHAG amino acid sequence has been substituted for by an amino acid with a reactive side chain, said amino acid with reactive side chain selected from the group consisting of cysteine, lysine, tyrosine, glutamate, aspartate, and arginine.

84. A polypeptide according to claim 83, wherein the SCHAG amino acid sequence comprises SEQ ID NO: 1, with the proviso that amino acid 184 of SEQ ID NO: 1 has been is substituted for by an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamate, aspartate, and arginine.

85. A polypeptide according to claim 84, wherein the SCHAG amino acid sequence comprises SEQ ID NO: 1, with the proviso that amino acid 2 of SEQ ID NO: 1 has been is substituted for by an amino acid selected from the group consisting of cysteine, lysine, tyrosine, glutamate, aspartate, and arginine.

86. A method of making a polymer comprising two or more regions with distinct function, said method comprising steps of:

(a) (i) providing a first polypeptide that comprises a SCHAG amino acid sequence and a first functional domain and

(ii) providing a second polypeptide that comprises a SCHAG amino acid sequence and a second functional domain that differs from the first functional domain,

wherein the SCHAG amino acid sequences of the polypeptides are capable of forming ordered aggregates with polypeptides identical to the first polypeptide or the second polypeptide;

(b) aggregating the first polypeptide by subjecting a composition comprising the first polypeptide to conditions favorable to aggregation of the first polypeptide into ordered aggregates, thereby forming a polymer comprising a region containing polypeptides that include the first functional domain;

(c) mixing a composition comprising the second polypeptide with the polymer formed according to step (b), under conditions favorable to aggregation of the second polypeptide with the polymer of step (b), thereby forming a polymer comprising the first region containing polypeptides that include the first functional domain and a second region containing polypeptides that include the second functional domain.

87. A method according to claim 86, wherein the SCHAG amino acid sequences of the first and second polypeptides are identical.

88. A method according to claim 86, wherein at least one of the first and second functional domains comprises an amino acid that comprises a reactive amino acid side chain.

89. A method according to claim 86, wherein at least one of the first and second functional domains comprises an amino acid sequence of a polypeptide of interest.

90. A method according to claim 86, further comprising a step of:

(d) mixing a composition comprising the first polypeptide with the polymer formed according to step (c), under conditions favorable to aggregation of the first polypeptide with the polymer of step (c), thereby forming a polymer comprising the first region containing polypeptides that include the first functional domain, the second region containing polypeptides that include the second functional domain, and a third region containing polypeptides that include the first functional domain.

91. A polymer fiber comprising two or more functional domains, formed according to the method of claim 86.

92. A method according to claim 86, further comprising steps of:

(a) (iii) providing a third polypeptide that comprises a SCHAG amino acid sequence and a third functional domain that differs from the first and second functional domains, wherein the SCHAG amino acid sequence of the third polypeptide is capable of forming ordered aggregates with polypeptides identical to the first polypeptide or the second polypeptide; and

(d) mixing a composition comprising the third polypeptide with the polymer formed according to step (c), under conditions favorable to aggregation of the third polypeptide with the polymer of step (c), thereby forming a polymer comprising the first region containing polypeptides that include the first functional domain, the second region containing polypeptides that include the second functional domain, and a third region containing polypeptides that include the third functional domain.

93. A composition comprising a fibril according to claim 31 attached to a solid support.

94. A composition comprising an ordered aggregate according to claim 49 attached to a solid support.

95. A composition comprising a polymer according to claim 67 attached to a solid support.

96. A composition comprising a fiber according to claim 76 attached to a solid support.

97. A living cell, said cell comprising:

(a) a first polynucleotide comprising a nucleotide sequence encoding a polypeptide that comprises a prion aggregation domain and a domain having transcription or translation modulating activity, wherein the living cell is capable of existing in a first stable phenotypic state characterized by the polypeptide existing in an unaggregated state and exerting a transcription or translation modulating activity and a second phenotypic state characterized by the polypeptide existing in an aggregated state and exerting altered transcription or translation modulating activity; and

(b) an exogenous polynucleotide comprising a nucleotide sequence that encodes a polypeptide of interest, with the proviso that the sequence encoding the polypeptide of interest includes a regulatory sequence causing differential expression of the polypeptide in the first phenotypic state compared to the second phenotypic state.

98. A living cell according to claim 97, wherein the cell further comprises a nucleotide sequence that encodes a polypeptide that modulates the expression level or conformational state of the polypeptide that comprises the prion aggregation domain.

99. A living cell according to claim 97, wherein the first polynucleotide comprises a nucleotide sequence encoding a polypeptide that comprises a prion aggregation domain fused in-frame to a nucleotide sequence encoding a translation termination factor polypeptide; and wherein the regulatory sequence comprises a stop codon that interrupts translation of the polypepide of interest.

100. A living cell, said cell comprising:

(a) a polynucleotide comprising a nucleotide sequence encoding a polypeptide that comprises a prion aggregation domain fused in-frame to a nucleotide sequence encoding a translation termination factor polypeptide; and

(b) an exogenous polynucleotide comprising a nucleotide sequence that encodes a polypeptide of interest, with the proviso that the sequence encoding the polypeptide of interest includes at least one stop codon that interrupts translation of the polypeptide of interest;

wherein the living cell is capable of existing in a first stable phenotypic state characterized by translational fidelity and substantial absence of synthesis of the polypeptide of interest and a second phenotypic state characterized by aggregation of the translation termination factor, reduced translational fidelity, and expression of the polypeptide of interest.

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